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NEPHROLOGY FORUM

Pharmacokinetic determinants of cyclosporine and prednisone in renal transplant patients

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Case presentation

An 18-year-old woman was admitted to a local hospital with acute renal failure one year after receiving a one-haplotype, parent-donated renal allograft. The patient's original renal failure had been due to chronic pyelonephritis. Over the first year after transplantation, she had had a stable medical course; her immunosuppressive medications gradually were tapered to prednisone, 12.5 mg/day; azathioprine, 50 mg/day; and cyclosporine (CSA), 100 mg twice daily. Over the year, the serum creatinine values had ranged from 1.4 to 1.6 mg/dl. Two weeks prior to admission, the serum creatinine rose slightly, to 2.6 mg/dl. The CSA levels were in the "therapeutic-range." Despite a urine culture that was positive for *E. coli*, she was not thought to have pyelonephritis; she was afebrile, the allograft was non-tender, the urinalysis revealed only 4 to 6 white blood cells/high-power field, and a renal sonogram was normal. A diagnosis of late acute transplant rejection was made, and she was given 3 days of prednisolone in intravenous pulses. The serum creatinine returned to a level of 1.5 mg/dl following this treatment. Shortly thereafter, however, she developed severe oroesophageal moniliasis. This superimposed illness was so severe that ketoconazole therapy was initiated, 200 mg orally twice daily. The cyclosporine dose was empirically reduced to 80 mg orally twice daily. The patient was discharged receiving these two medications as well as prednisone, 20 mg twice daily. Azathioprine was withheld because of the moniliasis.

Following four days of therapy with ketoconazole, the patient became increasingly weak. She was unable to easily ingest fluids or food, and she lost 3 to 4 pounds in the 4 days before being referred for admission to the UCSF Medical Center (Moffit Hospital). Table 1 contains details of the patient's clinical course and followup. Note particularly the extremely high cyclosporine trough levels on admission. The patient's acute renal transplant dysfunction responded promptly to a significant reduction of the oral cyclosporine dose. Eventually, the dose of cyclosporine needed by the patient was 10% of that needed prior to ketoconazole administration. The clinical course subsequent to cessation of ketaconazole and reinstitution of cyclosporine, 80 mg twice daily, has been uneventful; the serum creatinine has remained stable at approximately 1.4 mg/dl.

Discussion

DR. FELIX FREY (*Professor of Medicine and Nephrology, University Hospital of Berne, Inselspital, Berne, Switzerland*): The patient under discussion today illustrates well the practical implications of the altered kinetics of cyclosporine and prednisone in renal transplant patients. The inhibition of some microsomal hepatic P-450 isoenzymes by ketoconazole substantially decreased the catabolism of cyclosporine and prednisolone; this decreased catabolism in turn led to both excess immunosuppression, as evidenced by the delayed response of the moniliasis to the antifungal therapy, and to cyclosporine-induced renal dysfunction. Knowledge of the mechanisms accounting for altered kinetics of immunosuppressive agents allows the clinician to calculate more appropriate doses ("dose finding") in such patients. In this discussion, therefore, I will review the determinants of the pharmacokinetics of cyclosporine and prednisone, delineate the pharmacodynamic relevance of altered pharmacokinetics, and propose a strategy for optimal dosing in patients with abnormal disposition of these xenobiotics.

Cyclosporine

The gastrointestinal absorption of cyclosporine is slow and incomplete [1–3]. Peak concentrations in blood occur 1 to 8 hours after oral administration; the absorption half-life (assuming first-order absorption) ranges from 0.6 to 2.3 hours [1–4]. The values of systemic availability vary with the method used for measuring cyclosporine. In one of our studies in 58 renal transplant patients, high-performance liquid chromatography measurements of the systemic availability of cyclosporine were 25% lower than radioimmunoassay (RIA) measurements using a polyclonal antibody (30% ± 13% versus 41% ± 14%) [3]. This

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Table 1. Clinical and laboratory data from Patient 1

Date	Wt (*kg*)	Hct (%)	Serum creatinine (*mg/dl*)	BUN (*mg/dl*)	Prednisone (*mg/day*)	CSA dose (*mg*)	HPLC[a] CSA trough level (*ng/ml*)	Ketoconazole (*mg/day*)
Baseline	50.0	28	1.4–1.6	30	12.5	100 BID	174–196	—
6/19	50.0	—	1.5	—	40	80 BID	174	400
6/23	48.0	28	3.4	—	—	—	—	400
6/24	48.6	28	4.2	110	40	50 QD	—	400
6/25			Renal ultrasound (admission)—normal morphology + normal Doppler Renal scan—good uptake, slightly delayed excretion					
	—	26	3.6	113	40	Held	825	400
6/26	—	—	3.1	109	40	50	—	400
6/27	49.2	25	2.7	78	40	Held	866	400
6/28	—	27	2.6	59	40	Held	—	400
6/29	—	—	—	—	40	50 Q 3 days	—	400
7/1	48.8	—	2.4	50	40	—	190	400
7/6	—	—	2.2	—	40	50 Q 3 days	178	400
7/13	—	—	—	—	40	80 BID	—	400
7/16	—	—	1.9	—	40	80 BID	120	—
7/31	46.8	—	1.4	25	15	80 BID	112	—

[a] HPLC = High-performance liquid chromatography; the "therapeutic" trough level at one year post transplant ranges from 100 ng/ml to 250 ng/ml.

difference in bioavailability is due to the fact that a fraction of oral cyclosporine absorbed from the gut is converted during its first passage through the gut wall or liver into metabolites that react with the polyclonal antibody used in the RIA technique, but are not detected by the more specific high-performance liquid chromatography method.

Gastrointestinal absorption of cyclosporine depends on three factors. The first is the coadministration of food (Table 2). After an oral dose of cyclosporine, the area under the concentration-versus-time curve of cyclosporine is about 60% higher when the drug is given with food than without food [5]. The mechanism by which eating enhances the absorption of cyclosporine is unknown. It is likely, however, that it is related to gallbladder contraction following the intake of food because the second determinant of cyclosporine absorption is bile. Cyclosporine is lipophilic; a bile deficit therefore impairs its absorption. This phenomenon has been demonstrated in liver transplant patients by insertion of a T-tube into the common bile duct for external bile drainage. Clamping of the biliary T-tube greatly improves the absorption of cyclosporine; the delivery of bile into the gut likely is the cause of the increased absorption [6]. Preliminary investigations suggest that the exact location of the delivery of bile into the gut is crucial. In pediatric liver transplant patients with a Roux-en-Y biliary enterostomy, bile and cyclosporine do not mix until they reach the distal small gut, where cyclosporine is malabsorbed [7]. Only a negligible fraction of oral cyclosporine reaches the systemic circulation by the lymphatic vessels. The third determinant of cyclosporine absorption appears to be gastrointestinal function. Bone marrow transplant recipients with diarrhea (due to radiation therapy, graft-versus-host disease, or infectious enteritis), 20% to 30% of patients with inflammatory bowel disease, and patients with a short bowel syndrome all have lower-than-anticipated blood levels after receiving an oral dose of cyclosporine [8–11]. Metoclopramide increased the mean area under the blood concentration-versus-time curve of cyclosporine after oral dosing by 30% in 14 patients; gastric motility thus might be relevant for the absorption of cyclosporine [12]. Finally, the systemic availability of oral cyclosporine increases during the first weeks after kidney transplantation for unclear reasons [13].

Table 2. Factors affecting the kinetics of cyclosporine

Factor	Total-body clearance	Gastrointestinal absorption	Reference
Liver failure	↓[a]	↓	32, 55, 60
Bile deficit	→	↓	6
Diarrhea	→	↓	8–11
Food intake	→	↑	5
Renal failure	(↓)[b]	(↓)	55, 62, 63
Children	↑	→	32, 61
Elderly subjects	↓	→	55
Inhibitors of P-450	↓	(↑)	37–40, 43, 44, 47–50 51, 52
Calcium-channel-blocking agents (diltiazem, nicardipine, verapamil)			
Ketoconazole			
Erythromycin			
Inducers of P-450	↑	(↓)	30–33
Phenytoin			
Barbiturates			
Carbamazepine			
Rifampicin			

[a] ↓: Diminished clearance or absorption; →: unchanged clearance or absorption; ↑: increased clearance or absorption.

[b] Unknown. The arrow in parentheses indicates the changes one might predict.

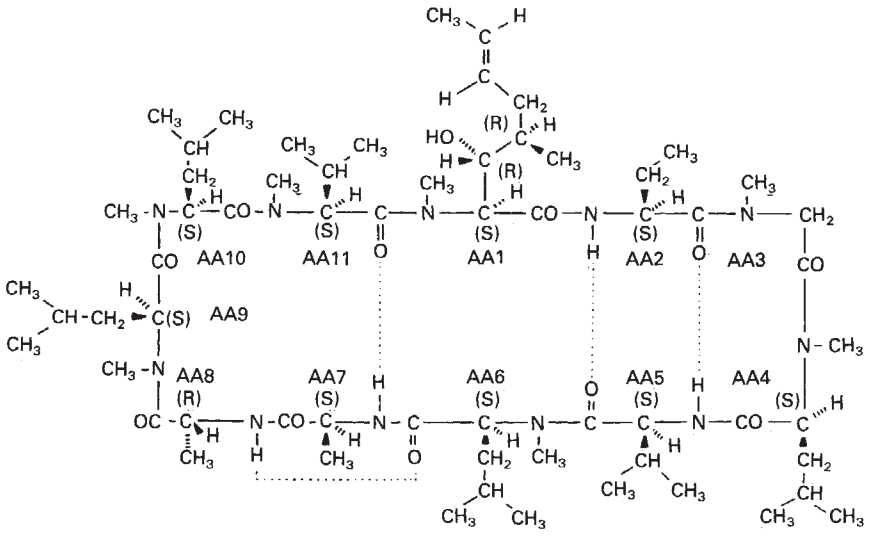

Fig. 1. *Structural formula of cyclosporine A.*

Cyclosporine undergoes extensive metabolism, predominantly in the liver [14], and metabolites are found in both bile and urine [15]. Virtually no unchanged cyclosporine is detectable in the urine. The biotransformation pathway of cyclosporine is similar in various animal species [16]. Whereas the cyclic oligopeptide structure of cyclosporine seems to be conserved, structural alterations during metabolism comprise mono- and dihydroxylation as well as oxidative N-demethylation (Fig. 1). In human hepatic microsomal preparations, the degradation of cyclosporine to its metabolites is NADPH dependent and can be inhibited by ketoconazole (as in the patient presented today), monoxide, and SKF 525A [14]; cyclosporine's metabolism thus might be mediated mainly through the multiple mono-oxygenase forms of cytochrome P-450.

At least 16 microsomal P-450 genes are expressed in humans [17]. The isoenzyme responsible for the major part of cyclosporine metabolism recently was identified by studying the metabolism of cyclosporine in rabbit liver microsomes [18]. Microsomes from phenobarbital-, beta-naphthoflavone-, triacyloleandomycin-, erythromycin-, or rifampin-treated and untreated rabbits were investigated; only microsomes from animals given the macrolide antibiotics (triacyloleandomycin or erythromycin, known to be specific inducers of form P-450 *3c*) exhibited a type-1 binding spectrum on cyclosporine addition and extensively metabolized the drug to all groups of derivatives [18]. The binding spectrum is an expression of a positive interaction between P-450 and its substrate. A linear correlation was found between cyclosporine oxidase activity and the specific content of P-450 *3c*. Antibodies to P-450 *3c* strongly inhibited cyclosporine oxidase activity of induced microsomes, whereas antibodies to other forms of P-450 did not. When highly purified forms of P-450 were assayed in a reconstituted system, only P-450 *3c* exhibited a type-1 binding spectrum on cyclosporine addition and extensively metabolized the drug to all derivatives [18]. The finding that P-450 *3c* is extensively involved in the formation of all derivatives of cyclosporine identified thus far is surprising, because it is well established that P-450 forms exhibit broad and overlapping substrate specificities [17].

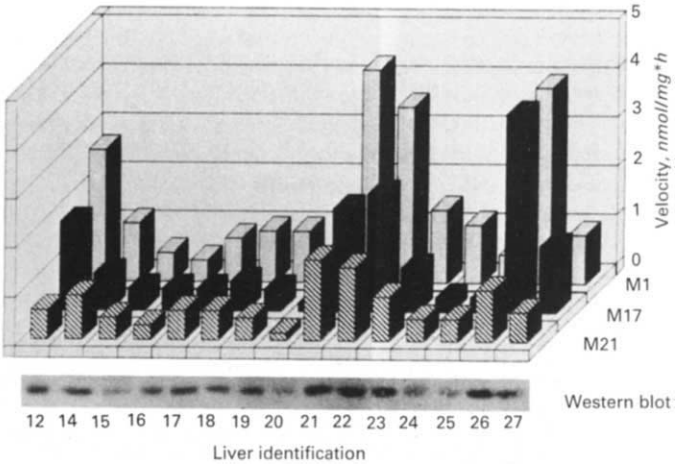

Fig. 2. *Formation of the three primary metabolites of cyclosporine in microsomes of 15 human livers from renal transplant donors and Western blots of microsomal protein (apparent mR 52000) recognized by monoclonal antibody 13-7-10.* The M1, M17, and M21 refer to individual metabolites of cyclosporine; the numbers at the bottom identify individual livers. (From Ref. 19.)

The P-450 *3c* found in rabbits appears to be homologous with the P-450III gene family in humans. Antibodies against P-450III in hepatic microsomes obtained from kidney transplant donors abrogated the formation of two monohydroxylated metabolites (M1 and M17) and of the demethylated metabolite (M21) [19]. These—M1, M17, and M21—are the three primary in-vivo metabolites of cyclosporine that are further metabolized by subsequent P-450-catalyzed reactions [20]. The conclusion that the P450III gene family in humans accounts for hepatic microsomal cyclosporine metabolism is supported by the correlation between the immunoquantitation of the protein recognized by the monoclonal antibody reacting with the human P-450III gene family and the metabolism of cyclosporine in the corresponding human hepatic microsomes (Fig. 2) [19].

The P-450 isoenzymes are inducible by specific xenobiotics

[17]. The increased amount and activity of isoenzymes observed following induction is attributable to an increased de-novo synthesis of protein, not to an impaired breakdown [21]. For some isoenzymes, it was shown that the increased synthesis is due to an increased transcription [22, 23]. Phenobarbital, one of the most carefully investigated inducers of P-450, induces several different mRNAs for the synthesis of various cytochrome P-450 isoenzymes [23]. How phenobarbital activates transcription of these genes and how the differential induction of some but not all P-450 isoenzymes is achieved is not known [24]. It has been proposed that induction is mediated via an intracellular receptor, as shown for polycyclic aromatic hydrocarbon- and steroid-responsive forms of cytochrome P-450 [25, 26]. Such a receptor has not been found, however. An alternative hypothesis is that the isoenzymes themselves are the receptors [24]. This concept is supported by the observations that first, many ligands and inhibitors of cytochrome P-450 are good inducers, and second, inducers reach high intracellular concentrations and are substrates of phenobarbital-induced cytochromes P-450 [27–29].

The relationship between the induction of hepatic P-450 isoenzymes and cyclosporine metabolism has been investigated in rats [30]. The administration of phenobarbitone together with cyclosporine decreased cyclosporine blood concentrations and prevented cyclosporine nephrotoxicity, assessed both biochemically and histologically. These findings in rats are in line with observations made in humans. In 6 healthy volunteers, administration of phenytoin for 9 days reduced the mean plasma concentration-versus-time curve ("area under the curve") after administration of oral cyclosporine by about 50% (Table 2) [31]. Cyclosporine was not given intravenously in this study, so it is unknown to what extent the reduction in blood levels was due to an increased metabolic clearance rate, first-pass metabolism, or decreased absorption of cyclosporine. Decreased concentrations of cyclosporine also were reported for a small number of patients treated with other agents known to induce hepatic microsomal enzymes, such as phenobarbitone, carbamazepine, rifampicin, and possibly isoniazid [32, 33]. Rigorous quantitative data concerning the effects of these agents on cyclosporine concentrations are not available.

The P-450 isoenzymes responsible for the formation of the primary metabolites of cyclosporine also are responsible for the metabolism of some calcium-channel blocking agents [29, 34]. These agents frequently are prescribed to renal transplant patients, because more than 70% of these patients are hypertensive. Moreover, there is some evidence for a slight protective effect of calcium-channel blocking agents on posttransplant acute tubular necrosis in patients treated with cyclosporine [35, 36]. This explains the interest in the study of the interaction between calcium-channel blocking agents and the kinetics of cyclosporine.

Cross-sectional analysis of dose and trough levels of cyclosporine in a total of 160 patients receiving or not receiving diltiazem revealed that a 20% to 50% reduction in the dose of cyclosporine was required to achieve comparable blood concentrations of cyclosporine when the patients were given diltiazem together with cyclosporine. This finding was confirmed in a crossover trial with 22 additional patients [37, 38]. Initiation and discontinuation of nifedipine had no effect on trough levels of cyclosporine in 22 renal transplant patients [37], whereas earlier individual case reports had suggested that verapamil and

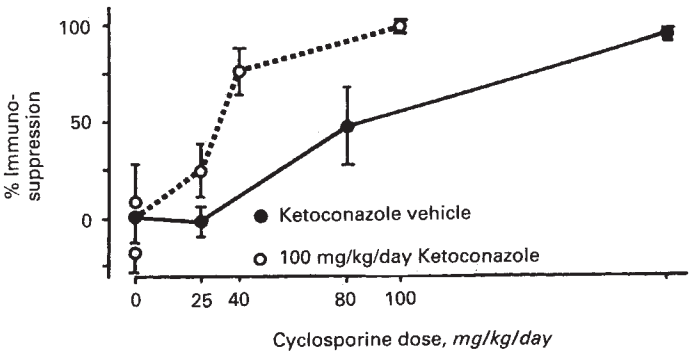

Fig. 3. *Cyclosporine dose-response curves for the suppression of delayed hypersensitivity response.* Dose of cyclosporine versus percentage of maximal immunosuppression is shown for animals treated concurrently with ketoconazole vehicle (●) or with 100 mg/kg/day of ketoconazole (○). Standard error of the mean is shown. Both curves originate at 0% immunosuppression. (From Ref. 50.)

nicardipine inhibited the metabolism of cyclosporine in such patients [39, 40]. It is not surprising that all calcium-channel blocking agents do not inhibit the metabolism of cyclosporine because, besides structural differences, local concentrations and affinity for the active site at the P-450 isoenzymes may differ among the various calcium-channel blockers [17].

In addition to the pharmacokinetic interaction, there is also a pharmacodynamic interaction between some calcium-channel blocking agents and cyclosporine. Verapamil inhibits T-cell activation and by that mechanism synergistically enhances the immunosuppressive effect of cyclosporine [41, 42]. To what extent this effect is clinically relevant is unknown, however. Practically, it is reassuring to know that reducing the dose of cyclosporine (to obtain an appropriate concentration of cyclosporine) in patients treated with verapamil might increase rather than decrease the overall immunosuppressive effect.

A similar pharmacodynamic and pharmacokinetic interaction exists between cyclosporine and ketoconazole (see also the discussion about steroids). Ketoconazole, an imidazole derivative, is a potent inhibitor of many P-450 isoenzymes, including the one responsible for the metabolism of cyclosporine, and it also inhibits many T-cell functions [43–46]. When ketoconazole is added to a cyclosporine regimen, increased concentrations of cyclosporine result in rats, mice, and humans [43, 44, 47–50]. These increased concentrations of cyclosporine in mice are due to a decreased metabolic clearance rate of cyclosporine [49]. The decreased metabolic clearance of cyclosporine is pharmacodynamically relevant [50]. The dose of cyclosporine that produces 50% of the maximal immunosuppression for cyclosporine is 2.5 times lower when cyclosporine is given together with ketoconazole in mice (Fig. 3). In addition, toxicity—as measured by the death of the animals (mostly because of infection)—is increased in animals receiving both drugs [50]. In rats the increased concentrations of cyclosporine that result from the coadministration of ketoconazole increased cyclosporine-associated nephrotoxicity [47]. Thus, the combination of abnormally high cyclosporine blood levels, decreasing renal function, and lack of response of the fungal infection in the patient presented are expected findings.

An interaction between erythromycin and cyclosporine has

Table 3. Strategies for dose finding

I	II	III
Dose ↓ ↑ Final therapeutic objective	Dose ↓ ↑ Biologic intermediate therapeutic objective	Dose ↓ ↑ Drug concentration

been reported repeatedly [51]. Because erythromycin inhibits the P-450 isoenzymes, it is likely that the increased concentrations of cyclosporine observed when erythromycin is given together with cyclosporine are due to a decreased catabolism of cyclosporine. However, an increased absorption of cyclosporine also has been suggested as the underlying mechanism [52]. Methyltestosterone, danazol, oral contraceptive steroids, and high doses of methylprednisolone all have been reported as increasing blood levels of cyclosporine [32, 51], but these scattered case reports are not conclusive.

Target concentration strategy for the dose finding of cyclosporine? There are three models for dose finding; unfortunately, none is ideally suited for dose finding of cyclosporine in clinical practice (Table 3). The most efficient method is adjustment of a drug dose according to the final therapeutic objective, as when a patient with headache is treated with paracetamol. This model is inappropriate for cyclosporine as it is whenever the failure to reach the therapeutic end point or the occurrence of side effects due to overtreatment is perilous. In such situations, the second method, namely the definition of an intermediate therapeutic objective, often improves the dose-finding process. The intermediate therapeutic objective can be either a target drug level or some biologic measure. An example of the latter is the determination of the prothrombin time in a patient receiving warfarin for pulmonary emboli; in this situation, titration of the dose according to the final therapeutic objective (prevention of emboli) and the side effect (bleeding) would be an inefficient way of adjusting the dose. Although conceptually it should be possible to define a biologic intermediate therapeutic objective for immunosuppressive therapy in renal transplantation, this is not yet feasible. Recently, measurements of cyclosporine level (as an alternative, intermediate therapeutic objective in renal transplantation) were advocated, a dose-finding strategy useful for many other drugs in modern clinical practice. In the patient under discussion, dosage adjustments, in fact, were made according to blood levels of cyclosporine. Thus, the question arises as to what extent cyclosporine fulfills the criteria for a meaningful application of such a "target concentration" strategy. Six criteria have been identified [53], only the first four of which are met by cyclosporine: (*1*) The drug must exhibit a wide interindividual variability in absorption and elimination. Cyclosporine certainly meets this criterion, as illustrated with regard to clearance in Figure 4 [54, 55]. (*2*) The time to reach the true therapeutic objective (years of successful renal function, in the case of cyclosporine) and the time when side effects appear should be long after the time of the therapeutic intervention. This is the case when cyclosporine is used to prevent renal allograft rejections. (*3*) The therapeutic index must be narrow. Most patients with trough whole-blood cyclosporine concentrations above 700 ng/ml (measured by the first-generation polyclonal antibody) experience side effects [56, 57]. The definition of a clinically efficacious concentration is more controversial, and no useful experimental data are available. Many clinicians agree that concentrations between 200 and 600 ng/ml are efficacious when cyclosporine is given either alone or with a low dose of prednisone. Thus, it is likely that the therapeutic index of cyclosporine is indeed narrow, an assumption also supported by a recent study in mice [50]. (*4*) The therapy must maintain a constant effect over a long time. Cyclosporine therapy also fulfills this criterion for a target concentration strategy. (*5*) The concentration of the drug in blood must be directly related to its effect. Concentration-effect curves between cyclosporine levels and inhibition of lymphocyte functions have been established in vitro. In clinical renal transplantation, however, even rough estimates between cyclosporine concentrations and the likelihood of rejection are missing. Thus, this prerequisite is not met for cyclosporine; future studies in this area are required. (*6*) The measurements of the drug concentrations should be specific and the individually measured blood concentration representative for the time-averaged concentration in a given individual. This is probably not the case for cyclosporine for two reasons. First,

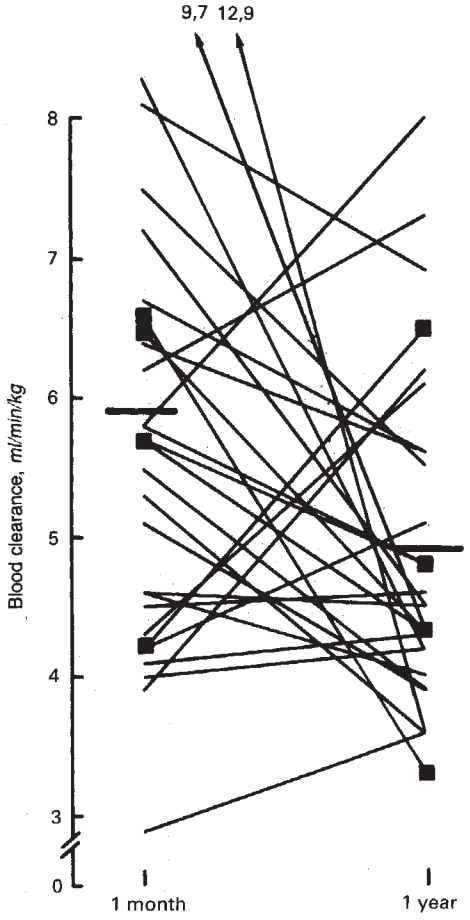

Fig. 4. *Blood clearance of cyclosporine (CyA) calculated by using whole blood concentrations assessed by high-performance liquid chromatography in stable renal transplant patients one month and one year after transplantation.* The clearance value declined in the course of the first year of successful renal transplantation in 19 of 28 patients. (From Ref. 54.)

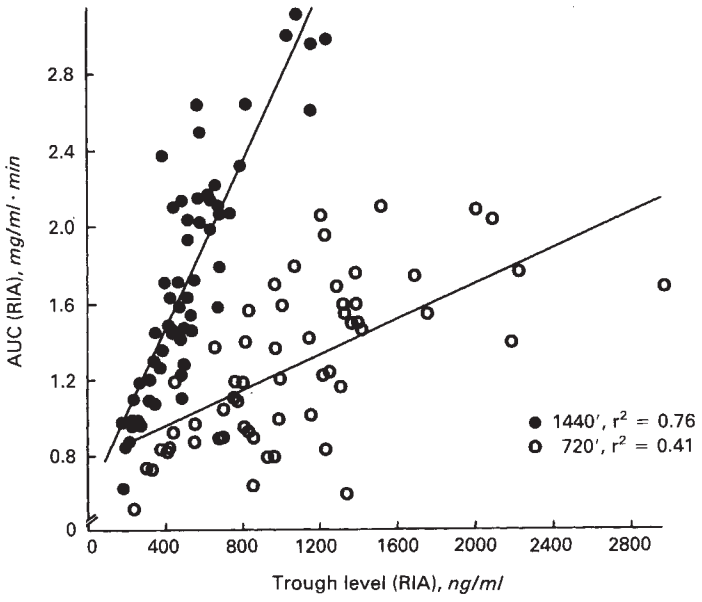

Fig. 5. *Relation between trough levels and areas under the curve (AUCs) assessed by radioimmunoassay after oral cyclosporine.* The relationship was established for trough levels assessed 720 and 1440 minutes after administration and the corresponding AUCs. Similar coefficients of determination (r^2) were obtained when the AUCs after oral or intravenous dosing were measured by high-performance liquid chromatography. The values of the r^2 were consistently lower when the trough levels were assessed 720 than 1440 minutes after administration. This finding indicates that trough levels measured at 720 minutes poorly reflect the exposure of the body to cyclosporine. (From Ref. 3.)

the various antibodies available detect different metabolites together with the parent compound, whereas the specific high-performance liquid chromatography method detects only the parent compound [58]. Because we do not know which of the compounds are responsible for efficacy and which for side effects, selection of the appropriate method for determining the concentrations remains problematic. Second, trough levels correlate poorly with the corresponding areas under the concentration-versus-time curves (AUC) for the corresponding dosing interval, indicating that trough levels offer only an incomplete reflection of the body's exposure to cyclosporine (Fig. 5) [3]. Because of this shortcoming, Grevel, Welsh, and Kahan recently proposed that cyclosporine be measured by determination of AUC values instead of trough levels, an approach not applicable at most institutions [59].

Thus cyclosporine does not fulfill these last two important prerequisites for a target concentration strategy. This deficiency explains the limited utility of using blood concentrations of cyclosporine for dose finding in clinical practice at present, unless they are used to detect a grossly abnormal disposition of cyclosporine (as in the patient discussed here). Despite these limitations, measuring blood concentrations of cyclosporine remains clinically relevant, especially in monitoring compliance, the most important variable of drug efficacy in outpatients.

Prednisolone/prednisone

Prednisolone is by far the most active immunosuppressive steroid when prednisolone or its in-vivo interconvertible 11-keto metabolite, prednisone, is administered [64]. Thus, kinetic studies should assess specific prednisolone concentrations in biologic fluids. Because patients treated chronically with a moderate dose of prednisone continue to secrete endogenous cortisone and cortisol [65], specific high-performance liquid chromatographic methods had to be developed to distinguish prednisolone both from prednisone and from its other metabolites and from endogenous glucocorticoids [66–68]. Prednisolone exhibits nonlinear binding to albumin and transcortin, with a free fraction ranging from less than 0.1 to 0.5 [69, 70]. The concentrations of these binding proteins are affected by hepatic and renal diseases and by the concomitant administration of estrogens [71, 72]. Given that the unbound species of prednisolone probably accounts for its biologic effect [73, 74], kinetic investigations have to focus on the unbound, rather than on the total, concentration of prednisolone.

Prednisolone and prednisone are interconvertible, and prednisolone is given intravenously as a water-soluble phosphate or hemisuccinate or tetrahydrophthalate prodrug. Thus, values of clearance and volume of distribution, derived from prednisolone concentration measurements in plasma, reflect only apparent values of these parameters. When calculated with reference to total prednisolone concentrations in plasma, the values of total-body clearance and volume of distribution increase with increasing doses of prednisolone administered, an observation made by our group and several groups of investigators, using different models for calculating the kinetic parameters [75–78]. Part of the dose-dependent clearance of prednisolone is explained by the concentration-dependent protein binding; that is, at high doses, the increased free fraction of prednisolone is reflected in a greater plasma clearance and apparent volume of distribution. The relevance of protein binding for the dose-dependency of prednisolone kinetics is supported by the observation that subjects exhibiting an abnormally high binding of prednisolone to plasma proteins (for example, women taking estrogen-containing oral contraceptive steroids) have decreased values of total-body clearance and volume of distribution of total prednisolone, whereas subjects exhibiting an abnormally low binding of prednisolone to plasma proteins (such as nephrotic patients) have increased values of total-body clearance and volume of distribution of total prednisolone [79–82].

In addition to the concentration-dependent plasma protein binding, the concentration-dependent clearance of unbound prednisolone also might account for part of the dose-dependent clearance of total prednisolone. This phenomenon was demonstrated by Legler et al, who showed that the total-body clearance of unbound prednisolone under steady-state conditions in normal volunteers is 30% higher after a high- than after a low-infusion rate [78]. The increase in total-body clearance of unbound prednisolone with increasing dose is probably due to an increase in renal and non-renal clearance [83]. The dose-dependent renal clearance cannot be explained by a dose-dependent impact of the corticosteroid on the glomerular filtration rate, because the ratios of the renal clearance of total or unbound prednisolone to the creatinine clearance (fractional renal excretion) are higher after a dose of 0.8 mg/kg than after a dose of 0.2 mg/kg of prednisolone [83].

After a low dose of 0.2 mg/kg of intravenous prednisolone, the ratio of the AUC for prednisolone over prednisone was 7,

Table 4. Factors affecting the kinetics of prednisolone/prednisone

Factor	Apparent total-body clearance	Gastrointestinal absorption	Reference
Liver failure	↓[a]	→	97
Chronic renal failure without a transplant	↓	? (↓)	92, 93
Renal transplant patients	↓	→	54
Hyperthyroidism	↑	↓	88
Inflammatory bowel diseases	→	↓→	87
Elderly subjects (≥65 yrs)	↓	→	90
Subject taking:			
Estrogen-containing contraceptive steroids	↓	→	80, 81 113, 114
Ketoconazole	↓	→	89
Carbimazole or methimazole	↑	?	120, 121
Phenytoin	↑	→	118, 119, 125
Barbiturates	↑	?	115, 126
Rifampin	↑	?	116, 130
Carbamazepine	(↑)	?	—
Some brands of enteric-coated prednisolone	→	↓	87
I.V. prednisolone tetrahydrophthalate	↑	—	84, 138

[a] Arrows are explained in Table 2.

whereas after a high dose of 7 mg/kg, this ratio was more than 66 in renal transplant patients [83, 84]. This finding suggests an apparent concentration-dependent interconversion between prednisolone and prednisone in favor of the former species. When very high doses of intravenous prednisolone or of oral prednisone (7 mg/kg) are given, prednisone concentrations appear to approach a maximum of only about 50 ng/ml to 60 ng/ml. The nonlinear relationship between the plasma concentrations of prednisolone and prednisone can be described by a Michaelis-Menten-type equation [78, 85]. The reason for the apparent shift of the plasma concentration ratios of prednisolone/prednisone towards prednisolone with increasing steroid doses is unknown [86].

As a consequence of the complex pharmacokinetics of prednisolone, accumulation of knowledge about the factors affecting the metabolism of this steroid has been slow [87]. The known mechanisms accounting for the altered kinetics of prednisolone are summarized in Table 4. Probably because of a short intestinal transit time, patients with inflammatory bowel disease [87] or hyperthyroidism [88] exhibit a decreased absorption of prednisolone. Some brands of enteric-coated prednisolone tablets are not absorbed or are only erratically absorbed in the gastrointestinal tract; such enteric-coated tablets therefore should not be administered to renal transplant patients [87].

The renal excretion of prednisolone is complicated and only partly understood. There is evidence that the renal clearance of total and unbound prednisolone increases with increasing dose [83]. After an intravenous dose of 0.8 mg/kg of prednisolone sodium phosphate, about 25% to 30% of the dose is recovered in urine as unchanged prednisolone, as opposed to only about 12% when an equimolar dose of oral prednisone is given [89, 90]. The percentage of a prednisolone dose excreted as prednisone is 2% to 5%. These higher values for the urinary excretion of unchanged steroid are too low to explain the 67% higher AUC values of unbound prednisolone after intravenous prednisolone in 6 chronically uremic patients than in healthy volunteers observed by Bergrem [91]. Oest et al found an identical mean AUC of total prednisolone in 16 patients on hemodialysis as in 6 healthy control subjects [92]. In the latter study, no unbound concentrations of prednisolone were measured. Thus, no definitive conclusion about the effect of renal function on the metabolism of prednisolone can be reached at present.

In patients with renal failure, a renal transplant, or hyperparathyroidism, and in subjects older than 65 years or those taking ketoconazole, the fraction of the dose of unchanged steroid recovered in urine is reduced [54, 88–90, 93]. The reduction is best explained in part by a diminished glomerular filtration rate (except in the case of hyperthyroidism) and in part by a reduced tubular secretion of prednisolone. The latter mechanism is suggested by the observation that in healthy volunteers the ratio between the renal clearance of unbound prednisolone and the creatinine clearance is about 2. This suggests tubular secretion of the steroid; this ratio was less than one in the other groups I mentioned.

Lewis et al found that 37% of the patients with serum albumin levels below 2.5 g/dl who were treated with prednisone experienced corticosteroid-related side effects, compared with 15% of those with serum albumin levels above 2.5 g/dl [94]. These investigators hypothesized that the low albumin levels simply might be a marker for impaired liver function (and thus impaired metabolism of prednisolone), or that hypoalbuminemia could directly cause increased free prednisolone levels, thereby resulting in an increased frequency of side effects. To examine this hypothesis, Bergrem and we administered intravenous prednisolone or oral prednisone to nephrotic patients with low protein concentrations and normal liver function [79, 82, 93]. Results showed decreased total, but normal unbound, concentrations of prednisolone in nephrotic patients when compared with normal volunteers [79, 82, 93]. Thus, the association between low albumin concentrations and increased incidence of side effects is *not* causal in nature, as suggested [94, 95], but rather exists because some patients have decreased albumin concentrations due to impaired liver function [96] and that impaired liver function is responsible for the diminished catabolism of prednisolone. A dosage adjustment of prednisolone based on plasma protein concentrations as previously proposed by Uribe and Go [95] is therefore wrong.

The systemic availability (that is, the ratio between the AUCs after oral and intravenous dosing) of prednisolone after administration of oral prednisone or prednisolone is not reduced in renal transplant patients [54, 77, 97, 98]. The values of renal and non-renal clearance of prednisolone are diminished, however. As a consequence, the mean total and unbound prednisolone concentrations after oral and intravenous dosing were higher by about 30% to 40% in 28 renal transplant patients treated with cyclosporine than were levels observed in an equal number of healthy volunteers (Fig. 6) [54]. The reduced prednisolone clearance was not due to the administration of cyclosporine [97, 99]. When the results from the same 28 patients obtained at one month and at one year after transplantation were compared, a decline in the non-renal clearance of prednisolone, concomitantly with a decline in the total-body clearance of cyclosporine and galactose [54], was observed (Fig. 6). Thus the activity of

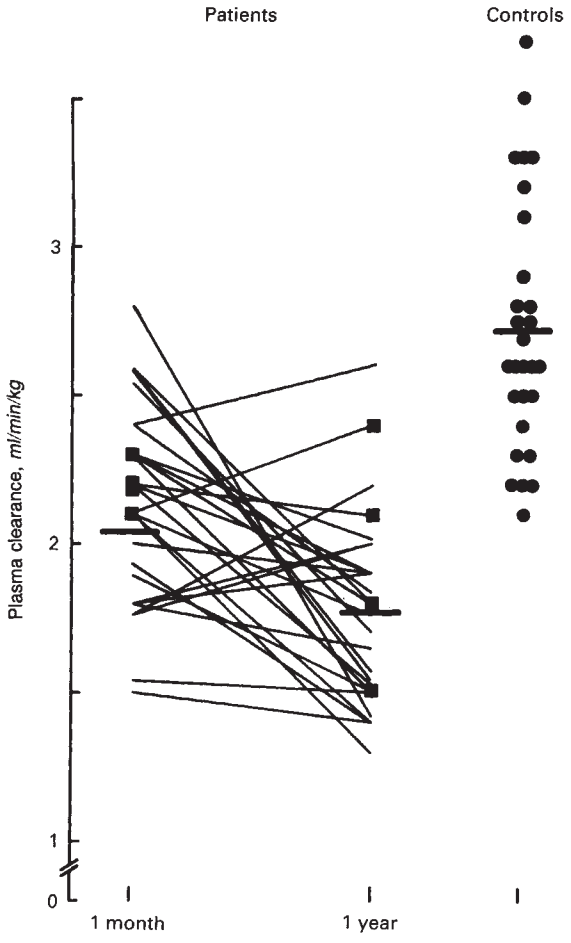

Fig. 6. *Plasma clearance of total prednisolone in 28 renal transplant patients at one month and at one year after transplantation and in 28 healthy control subjects.* The clearance declined during the first year of transplantation in 22 of 28 patients ($P < 0.001$). Squares = patients positive for hepatitis B markers; horizontal bars = the mean of the respective population. (From Ref. 54.)

both microsomal (clearance of prednisolone and cyclosporine) and cytosolic (galactose elimination capacity) liver enzymes declined in the course of the first year of successful renal transplantation. The decline in quantitative liver function was not due to inhibition of liver enzymes by drugs. The mechanism for the impaired liver function in these patients is unknown, but the observation is in line with the fact that more renal transplant patients die because of liver failure than do patients on dialysis or a population of normal subjects [54].

Prednisolone has been used as a model compound to investigate the impact of nonlinear plasma protein binding on hemodialysis clearance [100]. The hemodialysis clearance of total prednisolone was concentration dependent (increasing from 0.4 ml/min to 4.0 ml/min), while the clearance of unbound prednisolone was constant. With increasing total concentrations of prednisolone in plasma, a relatively larger fraction of the total plasma concentration was lost into the dialysate as a consequence of nonlinear plasma protein binding. Interestingly, the fraction of the dose of prednisolone administered that was lost in the dialysate did not rise as the administered dose of prednisolone was increased. This was because the fraction of the total-body prednisolone content entering the dialyzer was smaller as a consequence of the increased volume of distribution with the increasing dose of prednisolone. Thus, prednisolone exhibits concentration-dependent hemodialysis clearance but concentration-independent fractional removal of the dose. The fraction of the dose removed ranged from 7.0% to 17.5% during a 5-hour dialysis period, when the dose of prednisolone was administered just prior to the initiation of dialysis. Thus, for practical purposes, the removal of prednisolone by dialysis can be neglected.

The liver is the main organ for the catabolism of prednisolone and for the conversion of prednisone to the biologically active steroid prednisolone via reduction of the 11-oxo-group [86, 101]. This accounts for the studies of the impact of liver function on such interconversion. Despite the impaired apparent conversion of prednisone to prednisolone in patients with liver dysfunction, it is now clear that higher total and unbound prednisolone concentrations are observed after administration of oral prednisone in patients with impaired liver function [96]. This alteration occurs because the impaired conversion of prednisone to prednisolone is balanced by a reduced metabolic clearance of prednisolone in cirrhosis. The decreased metabolic clearance of prednisolone is associated with a decline in the fractional urinary excretion of 6beta-OH-prednisolone, indicating that the oxidative degradation of prednisolone is impaired, whereas the formation of other metabolites (mostly glucuronides) is preserved. This concept is in line with the established scheme that the glucuronidation of drugs is largely maintained in patients with advanced liver failure, while hydroxylation is impaired [102–104].

The daily administration of 200 mg of ketoconazole inhibits hepatic 6beta-hydroxylase activity, as assessed by the ratio of urinary 6beta-OH-cortisol/17-OH-corticosteroid, or by the fractional excretion of 6beta-OH-prednisolone (Fig. 7) [89]. The decline in activity of the 6beta-hydroxylase is associated with an impaired metabolic and renal clearance rate of prednisolone, an unaltered apparent systemic availability of oral prednisone, and an unchanged ratio of the AUCs of prednisolone/prednisone. The mean AUC of unbound prednisolone after oral prednisone increases by about 50% after ketoconazole. The 50% increased concentrations of free prednisolone in patients receiving prednisone and chronic low-dose therapy with ketoconazole are clinically relevant, because the increased immunosuppressive effect of prednisolone is enhanced by the known inhibitory effect of ketoconazole on T-lymphocyte functions [45, 46]. Thus the concomitant administration of ketoconazole and 40 mg of prednisone in the patient discussed here probably resulted in prednisolone blood levels one would usually observe in a patient given 80 mg of prednisone; direct measurements would have been of interest. The combination of increased prednisolone and cyclosporine concentrations no doubt explains the patient's increased susceptibility to infection. Whether other imidazole derivatives structurally related to ketoconazole and known to inhibit cytochrome P-450 oxidases [105, 106] have a similar impact on prednisolone metabolism is unknown. Cimetidine and ranitidine [106, 107], the macrolide antibiotic troleandomycin [108], and cyclosporine A [97, 99], all agents known to interfere with various mono-oxygenase systems in the liver, do not affect the metabolism of prednisolone.

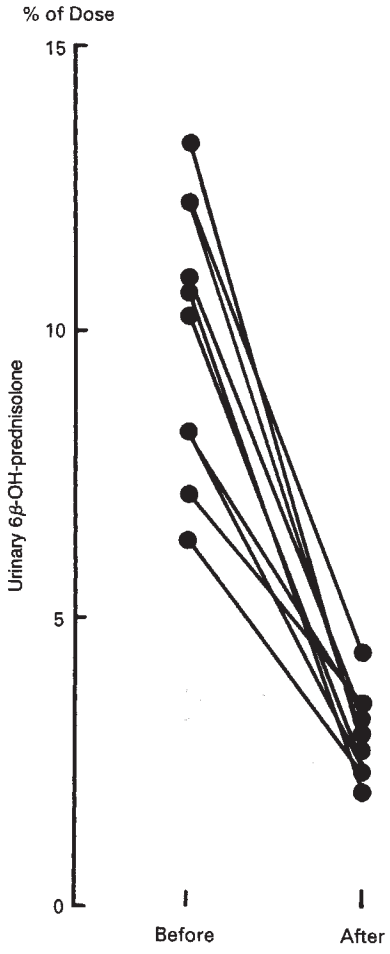

Fig. 7. *Urinary excretion of 6β-OH-prednisolone following intravenous prednisolone before and after ketoconazole administration.* (From Ref. 89.)

Two studies of cyclosporine [110, 111], disproved by more recent investigations and criticized for methodologic reasons [97, 99], had suggested an inhibitory effect of cyclosporine on prednisolone metabolism.

Ethinyl derivatives of estrogens render the P-450 system inactive, probably by covalent binding to prosthetic heme [112]. This effect explains the observation that in women taking oral contraceptive steroids, the fraction of the metabolic clearance of unbound prednisolone attributable to the formation of 6beta-OH-prednisolone is abnormally low [81]. As a consequence of the reduced catabolism, four independent groups of investigators found about 50% increased mean values of the AUCs of unbound prednisolone in women taking oral contraceptives concomitantly with prednisone or prednisolone [80, 81, 113, 114].

Several investigators have reported 30% to 50% lower plasma concentrations of total prednisolone after induction of the microsomal liver enzymes with phenytoin, barbiturates, carbamazepine, rifampin, or a combination of these agents [115–119]. During the administration of 300 mg/day of phenytoin for 6 to 8 days, protein binding, volume of distribution, and renal clearance of prednisolone were unchanged, whereas the metabolic clearance of unbound prednisolone increased by about 40%. Concomitantly there was an increased urinary excretion of 6beta-OH-prednisolone, a finding indicating increased microsomal liver enzyme activity [119].

In a cross-sectional study, Legler found about 50% lower concentrations of unbound and total prednisolone in women with Graves' ophthalmopathy treated with carbimazole or methimazole than in women who had undergone thyroidectomy for the same disease or in healthy female volunteers [120, 121]. All patients were euthyroid and were treated with prednisone for severe exophthalmos. Increased non-renal clearance of prednisolone in hyperthyroid patients recently was observed in a longitudinal study [88]. After treatment of hyperthyroidism, AUCs of total and unbound prednisolone after oral prednisone and intravenous prednisolone increased by about 40%. Induction or inhibition of microsomal liver enzymes changes the fractional excretion of 6beta-OH-prednisolone and the non-renal clearance of prednisolone in parallel [90, 119]. Interestingly, this relationship did not hold when the non-renal clearance of prednisolone was activated by thyroid hormones; that is, hyperthyroid patients had an increased non-renal clearance of prednisolone in the presence of a decreased fractional excretion of 6beta-OH-prednisolone [88]. This result indicates that thyroxine apparently inhibits some enzymes involved in the metabolism of prednisolone while stimulating other enzymes. The concept that thyroxine exhibits a differential effect on various liver enzymes is supported by animal and cell culture studies [122–124].

Evidence that altered kinetics of prednisolone are pharmacodynamically relevant

First, we have shown in in-vitro studies that plasma samples obtained from subjects treated with phenytoin and prednisone inhibit mixed lymphocyte cultures less than do plasma samples from the same subjects treated with prednisone only [125]. Similarly, the inhibition of mixed lymphocyte cultures after the same dose of prednisone was more pronounced in euthyroid than in hyperthyroid patients [88]. Second, in kidney and heart transplantation studies performed in rats treated with prednisolone or prednisolone plus phenobarbital, the organ survival time was shorter in the animals with, than in those without, induced microsomal liver enzymes (that is, in those receiving phenobarbital) [126]. Third, Wassner et al [127, 128] and Buffington et al [129] presented circumstantial evidence that an increased incidence of renal allograft rejection in humans treated with azathioprine and prednisone was attributed to anticonvulsants (barbiturates, phenytoin, carbamazepine) or rifampin (Fig. 8). Given that 6-mercaptopurine, the active moiety of azathioprine, is metabolized by xanthine oxidase, an enzyme not inducible by the aforementioned xenobiotics, these results suggest that the rejection episodes were mediated via enhanced catabolism of prednisone. In a prospective study, Öst et al analyzed the relationship between the metabolic clearance of total prednisolone after an oral test dose and the outcome of the renal allograft [92]. These authors found that the frequency of graft loss was higher in the group of patients with high than in the group with low clearance values. Fourth, the addition of rifampin to the drug regimen of prednisolone-treated patients with asthma produced a measurable deterioration in the patients' clinical status and an improvement in the adrenocortical

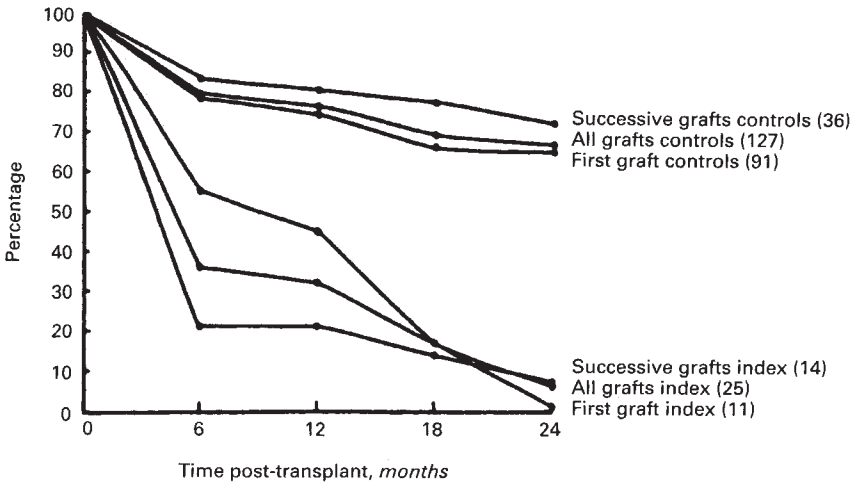

Fig. 8. *Actuarial survival rates of grafts in patients taking anticonvulsant medication (index patients) versus grafts in control patients.* (From Ref. 128.)

response to exogenous ACTH [130, 131]. Similar therapeutic failures of steroid therapy have been reported for patients with the nephrotic syndrome or rheumatoid arthritis after the administration of rifampin or barbiturates [117, 132].

When estrogens are given together with prednisone, the increased unbound glucocorticoid concentrations most likely account for the increased glucocorticoid effects. These are: (*1*) increased inhibition of granuloma formation by hydrocortisone in guinea pigs [132]; (*2*) increased glucosuric effect of prednisolone in patients with diabetes [134]; (*3*) reduction of the requirements of corticosteroids in various chronic inflammatory skin diseases in an uncontrolled clinical setting [133]; and (*4*) increased area under the inhibition-versus-time curve of mixed lymphocyte reaction, a time-averaged measure of the immunosuppressive effect after a dose of prednisone [135] in healthy women taking oral contraceptives [73].

After an intravenous dose of prednisolone phthalate, the mean AUC of unbound prednisolone was only 45% of that obtained after an equimolar intravenous dose of prednisolone phosphate in 10 renal transplant patients [84]. These kinetic differences were of biologic importance as evidenced by comparing the effect of these two prodrugs on the inhibition of mixed lymphocyte cultures, of lymphocyte interleukin 2 release, or of the changes in the OKT4/OKT8 ratio in peripheral blood [136].

Target concentration strategy for the dose finding of prednisolone? Prednisolone fulfills several prerequisites for a "target concentration" strategy. First, there is a larger interindividual than intraindividual variability in prednisolone elimination (Fig. 6) [54, 137]. Second, both prevention of allograft rejection and serious side effects (osteopenia, aseptic bone necrosis, cataract, infection) occur long after the point when one chooses the dose of prednisolone. Third, the therapeutic index of prednisolone is low. Fourth, the immunosuppressive effects of prednisolone are directly related to its plasma concentration [86]. Fifth, prednisolone is given to sustain a constant effect over a long time in renal transplant patients. Sixth, sensitive and specific assays for measuring prednisolone concentrations and knowledge of average values of kinetic parameters are available. Thus prednisolone meets many of the criteria necessary for the target concentration strategy; yet such a strategy is not applied at present for the following reasons: First, unbound prednisolone concentrations are the relevant concentrations to measure, but a suitable assay method is not available in most laboratories. Second, because of the short half-life of prednisolone, steady-state levels are not obtained at the usual daily dosage (<50 mg/day). Thus either the entire AUC or a single concentration in a plasma sample collected at a well-defined time point after dosing would have to be analyzed. In addition, the short half-life diminishes the utility of plasma concentration measurements as a tool for the assessment of compliance. Third, prednisone is administered concomitantly with other immunosuppressive agents, making the assessment of the efficacy of each agent, and therefore the definition of target concentrations, difficult. Thus the target concentration strategy cannot be applied for prednisolone.

How can the knowledge concerning variable prednisolone metabolism be used for dose adjustment in clinical practice? We tentatively propose the following strategy [87]: *First*, without considering the metabolism of prednisolone, decide what dose would usually be prescribed to a given patient with a certain disease. This decision should be based on results from the literature and from the clinician's individual experience.* *Second*, consider whether the patient in question exhibits specific features known to alter the metabolism of prednisolone; if so, prescribe a modified dose according to the following rough quantitative guidelines. *Third*, after having decided about the initial dosage regimen, base further decisions entirely on desired and undesired effects. Kinetic reasoning is warranted only when factors known or suspected of influencing the metabolism or the absorption of the steroid change subsequently. In the patient discussed above, for instance, when a maintenance dose

* Note, it is probably unwise to consider features modulating the metabolism of prednisolone in every patient with a certain disease state, because in the decision for the à priori dose based on previous experience, the impact of such metabolic abnormalities have already implicitly been included [87].

of 40 mg of prednisone was considered adequate, that dose might have been reduced to 20 mg after the addition of ketoconazole.

The following factors influence the biologically relevant unbound concentrations of prednisolone and might be relevant for rational prescription of prednisone in clinical practice (Table 4):

(*a*) liver failure: increased prednisolone concentrations by 100% to 300% (depending on the residual liver function) should be expected;
(*b*) chronic renal failure without kidney transplant: 70% increased concentrations after intravenous dose; oral dose unknown;
(*c*) renal transplant patients: 70% increased concentrations after oral dose;
(*d*) estrogen-containing oral contraceptive steroids: 50% to 100% increased concentrations;
(*e*) ketoconazole: 50% increased concentrations;
(*f*) old age (>65 years): 70% increased concentrations;
(*g*) hyperthyroidism: 40% decreased concentrations; euthyroid patients with exophthalmos on carbimazole or methimazole: 50% decreased concentrations;
(*h*) induction of microsomal liver enzymes (phenytoin, barbiturates, rifampicin, carbamazepine): 30% to 50% decreased concentrations;
(*i*) inflammatory bowel disease: in some patients unpredictably reduced concentrations due to malabsorption;
(*j*) prednisolone sodium tetrahydrophthalate: 50% decreased concentrations; some brands of enteric-coated prednisolone: unpredictably reduced concentrations.

Conclusion

The patient we are discussing dramatically demonstrates the clinical importance of the pharmacokinetics of cyclosporine and prednisone in renal transplant recipients. She developed severe oroesophageal moniliasis following treatment with high doses of corticosteroids for an acute renal allograft rejection. Ketoconazole, given to treat the fungal infection, caused an inhibition of the microsomal liver enzymes responsible for the degradation of cyclosporine and prednisolone. As a consequence, the patient had excess immunosuppression with retarded recovery from the infection and exaggerated cyclosporine nephrotoxicity. A more pronounced reduction of the cyclosporine dose together with a reduction of the dose of prednisone must be considered in patients given ketoconazole together with prednisone and cyclosporine.

Renal function is an important determinant of the pharmacokinetics of many water-soluble drugs. Therefore, altered renal function is commonly considered for dose-finding in clinical practice. Changes in liver function, on the other hand, are rarely considered in prescribing medications despite the fact that disease state and concurrent therapy can tremendously increase or diminish the liver's capacity to metabolize xenobiotics, as exemplified by the inhibition of P-450 by ketoconazole in the patient presented. The percentage reduction of the metabolic clearance of metabolized drugs in patients with severely impaired liver function can be comparable to the percentage reduction of the renal clearance of drugs excreted by the kidneys in patients with end-stage renal disease. Thus one might anticipate that taking into account the factors responsible for altered hepatic metabolism might improve our ability to prescribe metabolized drugs in the future, just as our consideration of renal function improved the dose-finding process for water-soluble xenobiotics in the past.

Questions and answers

DR. JOHN T. HARRINGTON (*Chief of Medicine, Newton-Wellesley Hospital, Newton, Massachusetts*): I understand that erythromycin causes induction of the P-450 system but that the enzymes induced are somehow immature. Could you please clarify the effects of erythromycin?

DR. FREY: Macrolide antibiotics such as erythromycin are potent inducers of P-450 isoenzymes [17, 18]. These isozymes are not immature but function normally. Erythromycin not only has the capacity to induce microsomal liver enzymes, but it is also a potent inhibitor of some P-450 isozymes. As a consequence of the inhibition of microsomal liver enzymes, cyclosporine A concentrations increase when erythromycin is added to cyclosporine A therapy in renal transplant patients [51].

DR. HARRINGTON: Dr. Frey, you commented at the onset of your discussion that it would be desirable to have an intermediate biologic objective for cyclosporine. Could you tell us what you think needs to be done to identify such intermediate biologic objectives and what would be meaningful parameters to be investigated for this purpose?

DR. FREY: The definition of an intermediate therapeutic objective for immunosuppressive therapy is not only desirable for cyclosporine A dosing but also for dose-finding of all other immunosuppressive agents. Such a measure would be desirable, especially in patients given a combination of drugs, in many transplant patients. The putative intermediate therapeutic objective should be of predictive value for the final therapeutic objective, which is absence of allograft rejection. Because the immunologic network of allograft rejection has not been completely elucidated, it is difficult for one to make a rational decision concerning the definition of a parameter reflecting overall immunosuppression. It is conceivable that measurements of soluble cytokines might be useful for that purpose in the future. The fact that not all the elements of the immunologic network have been discovered does not preclude that one might find a simple measure reflecting overall immunosuppression; as is well known, the prothrombin time, an extraordinarily useful intermediate therapeutic objective for anticoagulation with coumadin, was discovered long before the complex network of blood coagulation was entirely understood.

DR. MARTIN COGAN (*Chief, Division of Nephrology, Veterans Administration Hospital, San Francisco, California*): You suggested that cyclosporine concentration measurements are useful in assessing compliance and in the setting of complex pharmacologic interactions. Are cyclosporine measurements of any practical use in the more common clinical situations, such as a decrease in glomerular filtration rate in the posttransplant period?

DR. FREY: As I already mentioned, the methods used for measuring blood concentrations of cyclosporine do not assess the total concentrations of the biologically relevant cyclosporine metabolites. Therefore, one might predict that the utility of cyclosporine blood concentration measurements is of limited value when compliance or absorption or metabolism of cyclosporine is not grossly abnormal. With respect to the immediate

posttransplant period, absorption and metabolism of cyclosporine A can be quite variable. In addition, during that time period, many patients are not yet at steady state as far as cyclosporine A concentrations are concerned. Therefore, frequent monitoring of blood concentrations of cyclosporine A during the posttransplant period might be appropriate. Please note, however, that no formal trial has been set up to demonstrate unambiguously the clinical utility of such measurements.

DR. FLAVIO VINCENTI (*Professor of Medicine, University of California, San Francisco*): What should the clinician do when faced with very low trough levels of cyclosporine early after transplantation?

DR. FREY: First, increase the dose of cyclosporine A. If this does not increase the blood concentrations, it might be reasonable to administer cyclosporine A intravenously for some days until the absorption of cyclosporine A is normalized. Systemic availability of oral prednisone is less of a concern, so it might also be reasonable to increase the dose of prednisone for some days to have sufficient immunosuppression. I would not recommend a formal pharmacokinetic assessment in the early posttransplant period, because the parameters derived from such measurements might not be of predictive value for the kinetics in the same patient later.

DR. LESLIE Z. BENET (*Chairman, Department of Pharmacy, University of California, San Francisco*): If increased gastrointestinal transit, that is, diarrhea, decreases cyclosporine absorption, why does metoclopramide increase cyclosporine area under the curve [12]?

DR. FREY: Metoclopramide is known to stimulate the gastric emptying of isotype-labeled solid and liquid meals and has been shown to increase the bioavailability of other drugs. The mechanism for the increased bioavailability of xenobiotics following the administration of metoclopramide is, to the best of my knowledge, unknown.

DR. BENET: Dr. Frey, you already mentioned that the administration of food increases the cyclosporine A concentrations in blood after oral dosing. We have recently investigated the pharmacokinetics of cyclosporine A in healthy subjects following administration of cyclosporine both orally and intravenously without and with high-fat meals. Our findings corroborate previous studies in that the bioavailability of cyclosporine was estimated to be 21% and 79%, when administered without and with a high-fat meal, respectively. Surprisingly, blood and plasma clearance of cyclosporine A increased after the administration of a high-fat meal. The most likely interpretation for that finding is that changes in plasma lipid composition might affect volume of distribution and clearance of cyclosporine A [138].

It was suggested that many patients exhibit a second peak at 8 to 10 hours following oral dosing. Also, in some patients a second peak of immunosuppressive effects has been found. Can you confirm these observations?

DR. FREY: We have assessed the AUC of cyclosporine A (0 to 24 hours) in more than 80 instances. The blood concentrations were determined by radioimmunoassay and high-performance liquid chromatography. We cannot confirm the finding of a second peak by our physicochemical measurements. Some of the metabolites of cyclosporine A exhibit immunosuppressive activity. The kinetics of these metabolites might be different from the kinetics of the parent compound, and some of the metabolites might not be assessed by the physico-chemical methods used. Therefore it is conceivable that in some patients a second peak of immunosuppressive effect was present, despite the absence of such a peak, when cyclosporine was measured by radioimmunoassay or high-performance liquid chromatography.

DR. BENET: You used a specific assay to measure cyclosporine A in the patient discussed here. Would you please comment on the activity of cyclosporine A metabolites? Which ones would you expect to be present in greater concentrations after ketoconazole dosing?

DR. FREY: The administration of ketoconazole might change the pattern of cyclosporine A metabolites generated and alter the concentration ratios between these metabolites and the parent compound. These ratios have not yet been determined, so the interpretation of cyclosporine A measurements in patients taking ketoconazole is difficult. Assuming that the situation in mice is comparable to that in humans, it is likely that the increased concentrations of cyclosporine A measured by high-performance liquid chromatography are representative measures for the increased immunosuppressive activity after the administration of ketoconazole together with cyclosporine A. Indeed, Anderson and coworkers found that the blood concentrations of cyclosporine A doubled after the addition of ketoconazole and that these higher cyclosporine A concentrations were associated with a proportional reduction in the dose of cyclosporine A required to obtain the same immunosuppressive effect in vivo [49, 50].

DR. WILLIAM J.C. AMEND, JR. (*Professor of Medicine, University of California, San Francisco*): Dr. Kahan of Houston suggests that pretransplant evaluation of cyclosporine kinetics is important for posttransplant cyclosporine dosing. How do you react to this strategy?

DR. FREY: The basic assumption of the strategy is that the absorption and metabolism of cyclosporine A are independent of renal function and of the type of renal replacement therapy. This might be true for many but not for all patients. In addition, there is a practical limitation of that approach for most institutions: in many transplant centers it is not feasible to perform a complete pharmacokinetic assessment immediately before transplantation.

DR. AMEND: Are there any measures similar to HbA1c for glycemic control that could determine longer-term cyclosporine dose compliance?

DR. FREY: That is an interesting thought. Unfortunately, we don't know whether cyclosporine A is covalently bound to some plasma or cellular constituents, as is the case for other drugs [139].

DR. JOHN GAMBERTOGLIO (*Professor of Pharmacy, University of California, San Francisco*): Ketoconazole affects the metabolism of cyclosporine A. Do other antifungal agents also interfere with the metabolism of cyclosporine A or prednisolone?

DR. FREY: Whether amphotericin B or flucytosine affects the disposition of cyclosporine A or prednisone is not known. There are preliminary data indicating that fluconazole might interfere to a lesser degree than does ketoconazole with the metabolism of cyclosporine A [140, 141].

DR. CARLOS STEMPEL (*Renal Fellow, University of California, San Francisco*): First and coworkers demonstrated that the

addition of ketoconazole reduced the amount of cyclosporine required to achieve the same trough levels of cyclosporine and suggested that this might be a way to lessen the cost of organ transplantation [142]. Dr. Frey, could you comment on whether such a strategy is appropriate?

DR. FREY: The patients in that study were treated with prednisone, cyclosporine, ketoconazole, and azathioprine. Renal transplant patients usually have hypertension and thus require other drugs also. With the regimen proposed, most renal transplant patients might end up taking at least 6 drugs, a situation that jeopardizes compliance.

One reason for prescribing only a limited number of drugs to a given patient is to enable the clinicians to relate drug and effect. This might be difficult when ketoconazole is added, because ketoconazole itself is immunosuppressive and diminishes the catabolism of prednisolone and methylprednisolone and so might increase, unpredictably, the immunosuppressive effect [46, 89, 143]. Another concern is ketoconazole-associated liver injury; renal transplant patients exhibit impaired liver function and are at increased risk of death from liver failure.

As First et al showed, the variability of pharmacokinetic parameters for cyclosporine increases after the addition of ketoconazole [142]. The range of the dose of cyclosporine used to achieve the same blood concentrations of cyclosporine increased from 3- to 4-fold, the range of the C_{max} from 5- to 11-fold, and that of the "mean residence time" from 6- to 15-fold. This makes dosage adjustment more difficult in clinical practice.

The introduction of cyclosporine has indeed increased the costs of immunosuppression [144]. The overall cost-benefit impact still might be favorable, however. The addition of ketoconazole to cyclosporine might diminish the positive benefits to patients that the introduction of cyclosporine has yielded. For example, in First and colleagues' report, patients given ketoconazole were required to make two or three clinic visits per week for cyclosporine dose adjustment; one of the 17 patients treated with the combination had to discontinue taking ketoconazole because of gastrointestinal side effects, and two patients had rejections, probably related to ketoconazole, which were successfully treated with a 10-day course of OKT3 (cost, without inpatient charges, about US $4000 each). Without a controlled trial taking into account both the medical and the financial consequences, the prescription of a systematic drug interaction between cyclosporine and ketoconazole cannot be recommended. If First and colleagues wish to pursue their therapeutic strategy, it might be worthwhile reducing the liver's capacity to metabolize another drug of their triple therapy (azathioprine) by adding allopurinol [145].

MS. VICTORIA HALE (*Medical student, University of California, San Francisco*): You presented a very comprehensive list of compounds that affect the metabolism of prednisolone and cyclosporine. How do these compounds affect the metabolism of each other?

DR. FREY: Cyclosporine and prednisolone are metabolized by oxidases of the cytochrome P-450 system in the liver. Therefore it is reasonable for us to assume that those two drugs might interact at the level of those enzymes. As I already mentioned, cyclosporine A does not affect the metabolism of prednisolone. It is unknown whether prednisolone or methylprednisolone interferes with the metabolism of cyclosporine A.

DR. HENRY HULTER (*Associate Professor of Medicine, San Francisco General Hospital, San Francisco*): When oral prednisone or intravenous prednisolone is administered, prednisolone is found in three states in plasma: prednisolone bound to transcortin, prednisolone bound to albumin, and free prednisolone. What is the evidence that prednisolone bound to plasma proteins is biologically inactive?

DR. FREY: Women taking oral contraceptive steroids have more than a 100% higher concentration of prednisolone bound to transcortin than do normal subjects [73]. When these plasma samples containing various concentrations of prednisolone are incubated with mixed lymphocyte cultures and the degree of inhibition of the mixed lymphocyte reaction is quantified, higher total concentrations of prednisolone are required to produce half-maximal inhibition (EC_{50}) of the lymphocytes in women on oral contraceptive steroids than in controls. However, these concentration-response curves yield identical EC_{50} values when the unbound instead of the total concentration of prednisolone is plotted. This finding suggests that the higher transcortin-bound concentrations of prednisolone in women taking estrogens are biologically unimportant in this specific model. This conclusion is further supported by the observation that patients with low transcortin concentrations (nephrotic patients) have decreased EC_{50} values of total prednisolone when the same model (mixed lymphocyte reaction) is used [73].

DR. MICHAEL HUMPHREYS (*Chief, Division of Nephrology, San Francisco General Hospital*): The analysis of the concentration-response curves between unbound concentrations of prednisolone and inhibition of mixed lymphocyte cultures revealed a shift to the left in nephrotic patients when compared with healthy volunteers [73]. Such a shift might suggest the presence of immunosuppressive substances in plasma of nephrotic patients. For the concentration-response curves, the same baseline was utilized. Therefore, these endogenous immunosuppressive substances must simulate the glucocorticoid activity of unbound prednisolone. Is there more information about the mechanism of the interaction between an endogenous inhibitor and glucocorticoids?

DR. FREY: It has been known for more than 10 years that nephrotic serum contains inhibitory components for allogeneically or lectin-stimulated lymphocytes. The physicochemical properties of those agents have not been well defined, however. Because purification of those agents is still missing, more detailed interaction studies to assess the mechanism have not been possible until now.

The observation that the biologic activity of a certain drug level can be increased (and possibly also diminished) by endogenous compounds clearly indicates that a definition of a biologic measure for overall immunosuppression in a given patient would be of great help.

DR. D. UEHLINGER (*Nephrologist, Davis Medical Center, San Francisco*): Does ketoconazole also affect the metabolism of methylprednisolone, or is the interaction restricted to prednisolone?

DR. FREY: Ketoconazole inhibits the metabolic clearance rate of methylprednisolone in normal volunteers [143]. However, not all the P-450 isoenzymes involved in the metabolism of methylprednisolone are also biologically relevant for the

metabolism of prednisolone. For instance, macrolide antibiotics, such as troleandomycin and erythromycin, inhibit methylprednisolone metabolism but have no apparent effect on prednisolone metabolism. The concept that the various isozymes of the P-450 system have differential relevance for the metabolism of prednisolone and methylprednisolone is supported by the observation that induction of microsomal liver enzymes by phenobarbital, carbamazepine, and phenytoin revealed differences in the degree of stimulation of the catabolism of prednisolone and methylprednisolone in children [115].

DR. NICHOLAS V. HOLFORD (*Professor of Medicine, University of Auckland, Auckland, New Zealand*): It appears that you are proposing that if one could easily employ a target concentration strategy, you would recommend using that for prednisolone but probably not for cyclosporine. Some data seem to support the use of prednisolone, but I though you said the cyclosporine trials had yet to be done. Can you clarify that? Do you think that the trials should be performed for cyclosporine A, or do you think that there is no point in attempting to use a target concentration strategy for cyclosporine?

DR. FREY: It would be useful to perform a large trial to define more precisely the relationship between blood concentrations of cyclosporine and its efficacy and/or side effects in renal transplant patients. Such a study would be useful even if the result were negative, that is, in the absence of a relationship between the present, non-perfect, measurements of cyclosporine in blood and biologic effect.

DR. GAMBERTOGLIO: When prednisone is given, one can measure specifically unbound prednisolone in plasma. This is the only metabolite that exhibits biologic activity. Therefore, the most important prerequisite for a target concentration strategy, namely, that one can measure specifically the biologically relevant compound, is fulfilled. For cyclosporine, on the other hand, one cannot assess specifically the biologically relevant metabolites. Yet cyclosporine A measurements are widely used in clinical practice, whereas prednisolone measurements for dose-finding are virtually never considered. As Dr. Frey pointed out, this is largely due to the short half-life of prednisolone. It might be useful to consider prednisolone measurements by applying well-defined strategies for the timing of plasma sample collection to utilize the specific measurements available for prednisolone in clinical practice in the future.

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